PAPPA ELISA Kit

Catalog Number KA1281

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The PAPPA ELISA is an enzyme immunoassay for the quantitative in research measurement of Pregnancy associated plasma protein A (PAPP-A) in serum and plasma.

Background

PAPP-A is a protein produced by the developing placenta. Its concentration in the maternal blood increases rapidly after the 7th week of pregnancy. The measurement of PAPP-A in the first trimester of pregnancy has been reported as a useful marker in antenatal screening for Down Syndrome and other fetal aneuploidies. Reduced PAPP-A values in combination with maternal age, the measurement of free ß-HCG and the ultrasonic determination of nuchal translucency (NT) in pregnancy weeks 11 to 14 may detect up to 90 % of pregnancies with Down syndrom (reference 7).

The PAPP-A ELISA may be used for the risk assessment of Down´s syndrom (trisomy 21) in the first trimester of pregnancy. For the risk assessment of trisomy 21 and other fetal aneuploidies PAPP-A should always be measured in combination with other analytes (for example free ß-HCG and NT, see above) and a special software for the risk assessment of trisomy 21.
According to the IVD Directive (98/79/EC) both software and kits for the additional analytes must be suitable for trisomy 21 screening and CE-certified by a notified body, indicated by the identification number of the notified body on the CE-mark on software and kits.

Principle of the Assay

The PAPPA ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.
The microtiter wells are coated with a polyclonal anti PAPP-A antibody. An aliquot of patient sample containing endogenous PAPP-A is incubated in the coated well with assay buffer. After incubation the unbound material is washed off. In the second incubation step a sandwich complex is formed with a polyclonal anti PAPP-A antibody peroxidase conjugate. Having added the substrate solution, the intensity of color developed is proportional to the concentration of PAPP-A in the patient sample.

General Information

Materials Supplied

List of component

Components	Description	Volume / Qty.
Microtiterwells	Wells coated with anti-PAPP-A antibody (polyclonal).	12 x 8
Standard (Standard 0-5)	Concentrations: 0; 1; 2.5; 5.0; 15.0; 30.0 µg/ml Conversion: 1 mU/ml = 4.5 mg/l The PAPP-A Standards are comparable with NEQAS approved Reference material for Down Syndrome Screening (U/L, IRP 76/610) See „Preparation of Reagents"; contain 0.015% BND and 0.010% MIT as a preservative.	6 vials (lyophilized), 0.15 ml
Control (low and high)	For control values and ranges please refer to vial label or QC-Datasheet. Contains 0.015% BND and 0.010% MIT as a preservative.	2 vials (lyophilized), 0.15 ml
Assay Buffer	ready to use, contains 0.015% BND and 0.010% MIT as a preservative.	1 vial, 25 ml
Enzyme Conjugate 11X concentrate	complex containing horseradish peroxidase; see „Preparation of Reagents". Contains 0.03% Proclin, 0.015% BND and 0.010% MIT as a preservative.	1 vial, 1.5 ml
Conjugate Diluent	ready to use, Contains 0.03% Proclin, 0.015% BND and 0.010% MIT as a preservative.	1 vial, 14 ml
Substrate Solution	ready to use, Tetramethylbenzidine (TMB)	1 vial, 14 ml
Stop Solution	ready to use, contains 1 N acidic solution, Avoid contact with the stop solution. It may cause skin irritations and burns.	1 vial, 14 ml
Wash Solution	40X concentrated * BND = 5-bromo-5-nitro-1,3-dioxane MIT = 2-methyl-2H-isothiazol-3-one	1 vial, 30 ml

Note: Additional Standard 0 for sample dilution is available upon request.

Storage Instruction

When stored at 2-8℃ unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2-8℃. Microtiter wells must be stored at 2-8℃. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two months if stored as described above.

Materials Required but Not Supplied

- Equipment

✓ A microtiter plate calibrated reader (450 ± 10 nm)
✓ Calibrated variable precision micropipettes.
✓ Absorbent paper.
✓ Distilled or Deionized water
✓ Timer (60 min. range).
✓ Semi logarithmic graph paper or software for data reduction

Precautions for Use

- Precautions

✓ This kit is for in vitro diagnostic use only. For professional use only.
✓ All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
✓ Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
✓ The microplate contains snap-off strips. Unused wells must be stored at 2 ℃ to 8 ℃ in the sealed foil pouch and used in the frame provided.
✓ Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
✓ Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
✓ Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
✓ Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
✓ Allow the reagents to reach room temperature (21-26℃) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
✓ Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
✓ Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
✓ Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of

reagents or specimens may give false results.

- ✓ Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- ✓ Do not use reagents beyond expiry date as shown on the kit labels.
- ✓ All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- ✓ Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- ✓ Avoid contact with *Stop Solution* containing 1 N acidic solution. It may cause skin irritation and burns.
- ✓ Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
- ✓ TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- ✓ Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

- Limitations of Procedure

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

- ✓ Interfering Substances

  Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 30 mg/ml) have no influence on the assay results.
- ✓ Drug Interferences

  Until today no substances (drugs) are known to us, which have an influence to the measurement of PAPP-A in a sample.
- ✓ High-Dose-Hook Effect

  No hook effect was observed in this test up to 300 µg/ml of PAPP-A.

Assay Protocol

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

- ✓ Standards
  Reconstitute the lyophilized contents of the standard vial with 150 µl Aqua dest.
  Note: The reconstituted standards are stable for 2 months at 2-8°C.

- ✓ Control
  Reconstitute the lyophilized content with 150 µl Aqua dest. and let stand for 10 minutes in minimum. Mix the control several times before use.
  Note: The reconstituted control is stable for 2 months at 2-8°C.

- ✓ Wash Solution
  Add deionized water to the 40X concentrated Wash Solution.
  Dilute 30 ml of concentrated Wash Solution with 1170 ml deionized water to a final volume of 1200 ml.
  The diluted Wash Solution is stable for 2 weeks at room temperature.

- ✓ Enzyme Conjugate
  30 minutes before use dilute 1.0 ml of concentrated Enzyme Conjugate with 10 ml Conjugate Diluent.
  Note: The Enzyme Conjugate has to be prepared fresh 30 min. before use and cannot be stored longer than 24 hours. If more than one test run is performed, dilute only the quantity required for each test run.

Sample Preparation

Serum or plasma (EDTA-, heparin- or citrate plasma) can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

- ✓ Serum: Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

- ✓ Plasma: Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.

- ✓ Specimen Storage and Preparation: Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying. If EDTA plasma is stored at 2-8°C, it must be assayed within 48 hours.

Specimens held for a longer time (up to two months) should be frozen only once at -20℃ prior to assay. Thawed samples should be inverted several times prior to testing.

✓ Specimen Dilution: If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure. For the calculation of the concentrations this dilution factor has to be taken into account.
Example: (a) dilution 1:10: 10 µl Serum + 90 µl *Standard 0* (mix thoroughly)
(b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl *Standard 0* (mix thoroughly).

Assay Procedure

Each run must include a standard curve.
All standards, samples, and controls should be run in duplicate. All standards, samples, and controls should be run concurrently so that all conditions of testing are the same.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense 10 µl of each Standard, Control and samples with new disposable tips into appropriate wells.
3. Add 100 µl Assay Buffer into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for 30 minutes at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (400 µl). Strike the wells sharply on absorbent paper to remove residual water droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Dispense 100 µl diluted Enzyme Conjugate (see ”Preparation of Reagents”) into each well.
7. Incubate for 30 minutes at room temperature.
8. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (400 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add 100 µl of Substrate Solution to each well.
10. Incubate for 15 minutes at room temperature.
11. Stop the enzymatic reaction by adding 50 µl of Stop Solution to each well.
12. Determine the absorbance (OD) of each well at 450±10 nm with a microtiter plate reader.
    It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

- **Procedural Notes**

✓ All reagents and specimens must be allowed to come to room temperature before use. All reagents must

be mixed without foaming.

- ✓ Once the test has been started, all steps should be completed without interruption.
- ✓ Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- ✓ Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- ✓ As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Data Analysis

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted diluted or reported as > 30 µg/ml. For the calculation of the concentrations this dilution factor has to be taken into account.

- Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or Abnova directly.

- Example of Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard	Optical Units (450nm)
Standard 0 (0µg/ml)	0.18

Standard 1 (1µg/ml)	0.38
Standard 2 (2.5µg/ml)	0.56
Standard 3 (5µg/ml)	0.83
Standard 4 (15µg/ml)	1.44
Standard 5 (30µg/ml)	1.8

- Expected Normal Values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Pregnant women in the 1st trimester

238 samples of pregnant women in the 1st trimester have been measured with the PAPP-A ELISA.

The values are validated in comparison with a Gaussian distribution.

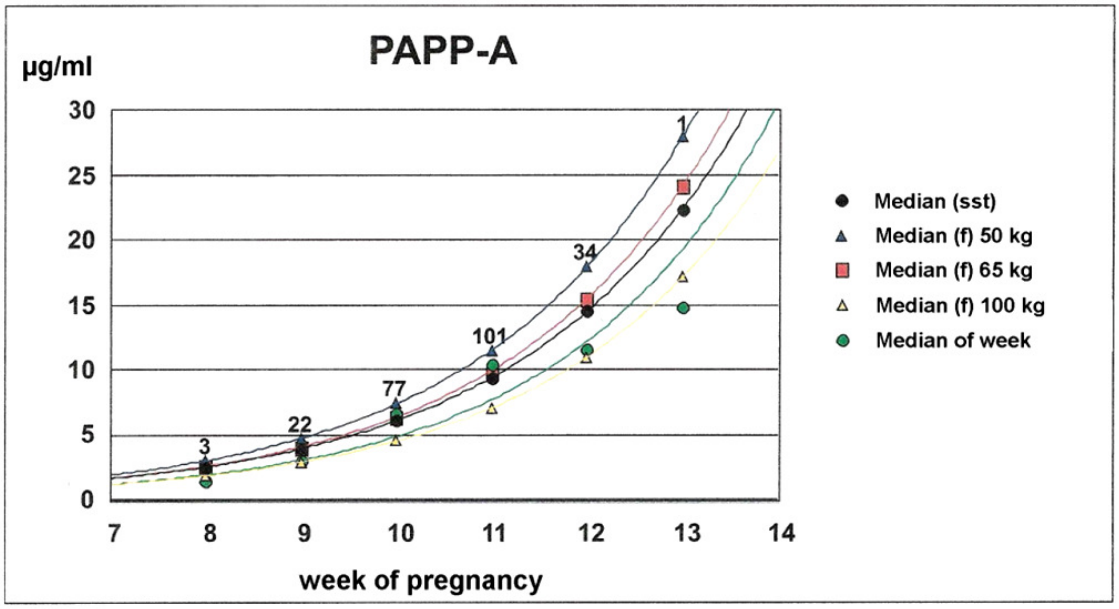
Consideration of body weight and day of gestation results in the following regression equation:

Median (f) PAPP-A = EXP (-2.12268 + 0.06324 * gestation day – 0.00979 * body weight).

If the values of the same 238 pregnant women are compared with the gestation day only (body weight not considered) the following weight independent regression equation is found:

Median (sst) PAPP-A = EXP (-2.705444 + 0.0618725 * gestation day).

In the following diagram and table the medians of function (median (f)) for **completed pregnancy weeks 8** to 13 have been calculated for three body weights (50 kg, 65 kg (mean body weight), and 100 kg). For comparison the medians were also determined manually (Median of week) and by using the weight independent regression equation (Median (sst)).



Completed week of gestation	day of gestation	Median(sst) [µg/ml] weight independent	Median (f) [µg/ml] weight 50 kg	Median (f) [µg/ml] weight 65 kg	Median (f) [µg/ml] weight 100 kg	Median of week [µg/ml]
8	59	2.57	3.06	2.6	1.88	1.5
9	66	3.97	4.77	4.1	2.92	3.0
10	73	6.12	7.42	6.4	4.55	6.7
11	80	9.43	11.55	10.0	7.08	10.5
12	87	14.55	17.99	15.5	11.03	11.6
13	94	22.43	28.00	24.2	17.17	14.9

Population and laboratory differences may lead to slightly different medians. Each laboratory should therefore determine and continuously update its own medians from its own patient collective. The regression equations and values in the table should be used as a guideline only. The calculation of medians and/or regression functions for the calculation of medians from own patient data bases should be performed with the applied trisomy 21 risk calculation software. Medians determined for the PAPP-A ELISA can not be used with assays of other manufacturers. Medians determined for PAPP-A assays of other manufacturers can not be used with the PAPP-A ELISA.

Use for Down Syndrom Screening

For risk calculation in prenatal screening PAPP-A concentrations are indicated as MOM (multiple of medians, MOM = Measured Concentration (PAPP-A) / Median PAPP-A).

In Down syndrom pregnancies the median of MOMs for PAPP-A are increasing during the first trimester and are not distinguishable anymore from normal pregnancies during the second trimester (reference 6, details see table). PAPP-A must therefore be measured in the first trimester of pregnancy (completed weeks 10–13).

Completed week of pregnancy	10	11	12	13	14-20
Median of MOM in pregnancies with Down Syndrom	0,34	0,42	0,50	0,58	1,11

Data from reference 6

For risk calculation of trisomy 21 not only PAPP-A but also other parameters like free ßHCG and nuchal translucency (NT) for the 1 st trimester and/or AFP, free Estriol and HCG for the 2 nd trimester have to be determined.

The use of these parameters for risk calculation of trisomy 21 requires a special software. According to the IVD Directive (98/79/EC) both software and kits for the additional analytes must be suitable for trisomy 21 screening and CE-certified by a notified body, indicated by the identification number of the notified body on the CE-mark on software and kits. The software must allow the calculation of medians from own patient measurements.

It is imperative to take into consideration additional factors, e.g. age of the woman, weight, ethnic group and smoker/non-smoker. An underestimation of the gestation age can lead to a falsely high calculated risk (false positive). To reduce this source of error, it is important to determine the gestation age as precisely as possible. Gestation age calculation from the last menstrual cycle inheres a high risk of variation.

Sonographic determination of the crown-rump length (CRL) or biparietal diameter (BIP) is recommended for the proper determination of the gestation age.

PAPP-A measurement in the course of a prenatal screening determines only a risk for trisomy 21.

For proof of trisomy 21 genetic determinations are required.

Performance Characteristics

- Assay Dynamic Range

  The range of the assay is between 0.133 µg/ml – 30 µg/ml.

- **Specificity of Antibodies (Cross Reactivity)**

  The antibody used for the PAPP-A ELISA is specific for human PAPP-A. There is no cross-reactivity to other species. o reaction is seen with normal human plasma.

- **Sensitivity**

  The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of *Standard 0* and was found to be 0.133 µg/ml.

- **Reproducibility**

✓ Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (µg/ml)	CV (%)
1	20	1.12	2.89
2	20	10.17	2.81

✓ Inter Assay Variation

The between assay variability is shown below:

Sample	n	Mean (µg/ml)	CV (%)
1	12	1.18	7.18
2	12	10.94	5.72

- **Recovery**

Sample	**Added Conc. (µg/ml)**	**Measured Conc. (µg/ml)**	**Expected Conc. (µg/ml)**	**Recovery (%)**
1	--	19.89	19.89	100
	1.25	10.94	11.20	97.7
	2.50	12.00	12.45	96.4
	7.50	17.66	17.45	101.2
	15.00	24.78	24.95	99.3

2	--	2.17	2.17	100
	1.25	2.44	2.34	104.3
	2.50	3.44	3.59	96.0
	7.50	9.00	8.59	104.8
	15.00	15.77	16.09	98.1

- **Linearity**

Sample	Dilution	Mean Conc. (µg/ml)	Recovery (%)
1	None	20.90	100
	1:2	10.30	98.5
	1:4	5.39	103.1
	1:8	2.61	100.0
	1:16	1.25	95.80
2	None	11.83	--
	1:2	5.80	98.1
	1:4	2.82	95.3
	1:8	1.45	98.1
	1:16	0.73	98.8

Resources

References

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Plate Layout

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